(54) Title: PHARMACEUTICAL COMPOSITIONS AND METHODS FOR COLONIC DELIVERY OF CORTICOSTEROIDS

(57) Abstract

Pharmaceutical compositions and methods are provided for the colon-specific delivery of corticosteroids (I). The corticosteroids are administered in the form of prodrugs which undergo reaction with enzymes produced by colonic microflora, thereby releasing the free drug.

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PHARMACEUTICAL COMPOSITIONS AND METHODS
FOR COLONIC DELIVERY OF CORTICOSTEROIDS

Description

10   Technical Field
        This invention relates to compositions and methods for
colon-specific drug delivery. More specifically, the invention
relates to colon-specific delivery of corticosteroid drugs using a
prodrug composition which, when ingested by a mammal, undergoes
15   reaction with enzymes which are produced by colonic microflora to
release the free drug.

        Background
        In recent years there has been increased emphasis on
20   ways to deliver or activate drugs at specific sites in order to
· reduce side effects and increase pharmacological response.
Implantable pumps, adhesive patches impregnated with drugs, vesicle--
enclosed drugs, and drug carriers have been proposed to achieve
site-specific delivery. Another approach has been to use prodrugs
25   (see Stella, V.J., and Himmelstein, K.J., J. Med. Chem. 23:1275-1282
(1980); Sinkula, A.A., and Yalkosky, S.H., J. Pharm. Sci. 64:181-210
(1975)) which, by virtue of their physicochemical properties, can
reach specific sites and then be converted to the active drug in
situ. The site-specific delivery of prodrugs administered to the
30   kidney, brain, breasts, the central nervous system, or topically to
the eyes or skin, have all been reported. In all of these cases,
the "parent" drug is released chemically or by specific enzyme or
enzymes present at the target site.
        The azo-reductase activity of the colonic microflora is
35   now known to activate certain sulfa drugs by reducing the azo-bond
present in such compounds. See Mandel, G.L., and Sande, M.A., in
"The Pharmacological Basis of Therapeutics," Sixth Ed. (A.G. Gilman,
L.S. Goodman, and A. Gilman, Eds.) MacMillan, New York, N.Y., (1980)
pp. 1106-1165. Also, the reduction of the azo-link between an
unabsorbed polymer and certain aromatic amines form the basis of a
recently developed colon-specific drug delivery system. See
Parkinson, T.M., Brown, J.P., and Windegard, R.E., U.S. Patent
4,190,716 (Feb. 26, 1980); and Brown, J.P., Appl. Enviro. Microbiol.
41:1283-1286 (1981).

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The present invention is specifically directed to methods and compositions for colon-specific delivery of corticosteroid drugs. The overall approach involves delivering the drug to the large intestine via a uronic acid carrier. The active drug is liberated by enzymes produced by the gut microflora, which

5   reside primarily in the large intestine. After drug absorption, much higher cecal and colonic tissue drug concentrations are possible than when such active agents are administered systemically at an equal dose. This approach to colonic delivery permits lower doses of corticosteroids to be administered while maintaining

10 efficacy.

The advantages of the invention are many. The development of a colon-specific delivery system for the administration of corticosteroid drugs will be useful to the many people who suffer from diseases, such as ulcerative colitis

15 (approximately 65 cases per 100,000 in the population as a whole) and Crohn's disease (approximately 30 cases per 100,000). The colon-specific corticosteroid delivery system of the invention is also useful in the treatment of radiation-induced colitis. The invention eliminates the complications which are frequently

20 encountered with the systemic administration of corticosteroids (including muscle wasting, osteoporosis, growth retardation and the like). Additionally, as noted above, the invention enables the use of far lower doses of corticosteroid drugs than previously possible; this is important, also, in reducing side effects. The invention

25 addresses the need in the art for a corticosteroid formulation which is orally active; it is well known that many corticosteroid drugs have little activity when administered orally. With the present prodrug compositions, however, even these drugs are rendered orally active for treatment of ulcerative colitis and Crohn's disease of

30 the colon. Finally, the invention addresses the need to deliver a corticosteroid not only to ascending and transverse colon, but also the descending and sigmoid colon. The distal colon is the primary site of inflammation in patients with ulcerative colitis and Crohn's disease of the colon. As the disease proceeds, it moves to the

35 proximal parts of the colon and increases in severity. Therefore, the invention should be useful to almost all individuals with ulcerative colitis and Crohn's disease of the colon regardless of the site of inflammation.

Previous efforts in devising a colon-specific corticosteroid delivery system have included administration via the enteral route using traditional enteric coatings, e.g., methylacrylic-methyl methacrylic copolymers. Such systems are described in G. Bogentoft et al., Acta Pharm. Suec. 20:311-314 (1983), M.J. Dew et al., Br. J. Clin. Pharmacol. 14:405-408 (1982)

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and P. Thomas et al., J. Pharm. Pharmacol. 37:757-758 (1985). These systems are predicated on the pH gradient that exists from the stomach to the small intestine and colon. Data from studies involving such coated corticosteroid formulations have indicated a considerable degree of variability of capsule breakage and drug

5   release (as noted in the Dew et al. and Thomas et al. publications, supra); more recently, it has been concluded that luminal pH in the gastrointestinal tract may not be used reliably and routinely to trigger release of drugs in the colon using enteric coatings. (P. Gruber et al., Adv. Drug Del. Rel. 1:1-18 (1987)).

10   Attempts have also been made to administer corticosteroids in prodrug form using a specific glycoside as the "disabling" moiety. One such effort is documented in PCT Publication No. WO84/04041, entitled "Colon-Specific Drug Delivery System," inventors Friend and Chang, and by Friend et al. in J. Med.

15   Chem. 27:261-266 (1985) and J. Med. Chem. 28:51-57 (1985). However, the efficacy of the formulations--which were based on D-glucose, D-galactose and D-cellobiose--was found to be limited; the uronic acid-based prodrugs which have now been developed and are described and claimed herein surprisingly display far greater efficacy at

20   lower doses than the earlier systems.

           In addition to the references discussed above, the following patents and publications relate to one or more aspects of the present invention:

           U.S. Patent Nos. 4,443,440 and 4,456,602 to Anderson et

25   al. describe amine-containing ester prodrugs of corticosteroids which are stated to be "solution stable" in vitro but labile and thus converted in vivo to the active parent drug. U.S. Patent Nos. 4,469,689 and 4,472,392 to Anderson et al. are similar but relate to sulfonate-containing ester prodrugs of corticosteroids, while U.S.

30   Patent No. 4,588,718 to Anderson et al. relates to carboxy-containing ester prodrugs of corticosteroids.

           U.S. Patent No. 4,221,787 to Bodor et al. describes esteramide prodrugs of corticosteroids formulated for delivery to the skin.

35   U.S. Patent Nos. 4,548,922 and 4,959,358 to Carey et al. describe pharmaceutical compositions for delivering a drug transmucosally, the compositions containing, as a permeation enhancer, a steroid optionally conjugated to an organic group such as a uronic acid.

Disclosure of the Invention

           Accordingly, it is a primary object of the invention to address the above-discussed needs in the art by providing a

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corticosteroid drug in a form which enables colon-specific drug delivery.

It is another object of the invention to provide a colon-specific prodrug comprised of a corticosteroid-sugar conjugate capable of being cleaved by enzymatic activity of colonic microflora
5   but not capable of being significantly hydrolyzed by endogenous enzymes produced by the mammalian host, thus enabling free drug to be released in the area of the colon following cleavage of the prodrug.

It is still another object of the invention to provide a
10   colon-specific prodrug comprised of a corticosteroid glucuronide capable of being cleaved at a rate in the colon such that the corticosteroid is released in the ascending, transverse, sigmoid, and descending colon.

It is yet another object of the invention to provide a
15   pharmaceutical composition containing the corticosteroid glucuronide prodrug in combination with a pharmaceutically acceptable carrier.

It is a further object of the invention to provide a method for delivering a corticosteroid drug to the colon of a warm-blooded host animal which involves orally administering a
20   corticosteroid drug in a form which enables colon-specific delivery of the drug.

It is still a further object of the invention to provide such a method wherein the corticosteroid is administered as a prodrug comprised of a corticosteroid glucuronide capable of being
25   cleaved by glucuronidase enzymatic activity of colonic microflora but not capable of being significantly hydrolyzed by endogenous enzymes produced by the mammalian host.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which
30   follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

The prodrugs of the present invention utilize a sugar moiety such as a uronic acid as the disabling moiety that
35   substantially prevents liberation or absorption of the free drug until the prodrug reaches the area of the colon. Colonic microfloral glycosidases then act upon the prodrug, cleaving it to release the drug in active form. The novel prodrugs comprise a corticosteroid drug bonded through the 21-hydroxyl group to the C_1 position of a sugar moiety such as β-D-glucuronic acid. The prodrugs may be represented by the structural formula

(I)   R - X - St

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wherein R represents a sugar moiety as will be described in further
detail below, "St" represents the corticosteroid moiety, and X is
oxygen or sulfur, preferably oxygen. The sugar moiety is thus
linked to the corticosteroid through an ether or thioether linkage.
As with the corticosteroid prodrugs described in U.S. Patent Nos.
5   4,469,689 and 4,472,392 to Anderson et al., the "parent"
corticosteroid of the prodrug may be represented as StOH wherein the
OH is located at the 21-position of the corticosteroid which may be
depicted as follows:

10

                        21 CH₂OH
                           |
        (II)   20 C = O
                           |
15   ╱17╲
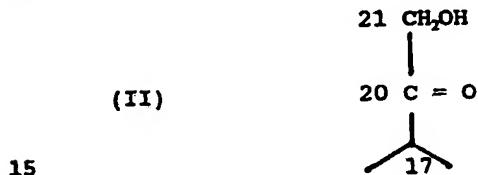
        Alternatively, a thiol may be present at the 21-
20   position, giving rise to a thioether linkage between the
corticosteroid and the sugar species.

Brief Description of the Figures
        Figures 1A-1C illustrate the specific activity
25   (nmol/mg/min) of hydrolysis of p-nitrophenyl-glucosidase (p-NP-glc)
and p-nitrophenyl-glucuronidase (p-NP-glrd) (substrate
concentration, 2.0 mM) in homogenates of the luminal contents from
various locations in the conventional rat (Figure 1A), the germ-free
rat (Figure 1B) and the acetic acid-induced colitis rat (Figure 1C).
30   Figures 2A-2C illustrate the specific activity
(nmol/mg/min) of hydrolysis of p-nitrophenyl-glucosidase (p-NP-glc)
and p-nitrophenyl-glucuronidase (p-NP-glrd) (substrate
concentration, 2.0 mM) in homogenates of the mucosal scrappings from
various locations in the conventional rat (Figure 2A), the germ-free
35   rat (Figure 2B) and the acetic acid-induced colitis rat (Figure 2C).
        Figures 3A-3C illustrate the specific activity
(nmol/mg/min) of hydrolysis of p-nitrophenyl-glucosidase (p-NP-glc)
and p-nitrophenyl-glucuronidase (p-NP-glrd) (substrate
concentration, 2.0 mM) in homogenates of the intestinal tissues
(muscle layer) from various locations in the conventional rat
(Figure 3A), the germ-free rat (Figure 3B) and the acetic acid-
induced colitis rat (Figure 3C).
        Figures 4A-4C illustrate the specific activity
(nmol/mg/min) of hydrolysis of dexamethasone-β-D-glucuronide

(substrate concentration, 0.5 mM) in homogenates of the intestinal contents (Figure 4A), mucosal scrappings (Figure 4B) and tissues (muscle layer; Figure 4C) from various locations in the conventional rat. Error bars are A.D.(n=3).

Figure 5 illustrates results obtained upon treatment of carrageenan-induced colitis in guinea pigs with dexamethasone-β-D-glucuronide.

Figure 6 illustrates colonic fluid absorption in rats with and without acetic acid (4%)-induced colitis, treated with either dexamethasone or dexamethasone β-D-glucuronide, orally.

Figure 7 illustrates in graph form the results of ulcerated surface area measurements for groups of rats as described in detail in Example 3.

Figure 8 illustrates in graph form the colonic fluid flow data obtained in Example 4, for budesonide and budesonide-β-D-glucuronide.

Figure 9 illustrates in graph form the results of ulcerated surface area measurements for groups of rats as described in detail in Example 4.

Modes for Carrying Out the Invention

Definitions:

Before the present compositions and methods are disclosed and described, it is to be understood that this invention is not limited to the administration of specific corticosteroid drugs, specific carrier materials or the like, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a corticosteroid" includes mixtures of corticosteroids, reference to "a carrier" includes mixtures of two or more carriers, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, the phrase "optional double bond" means that a double bond may or may not be present in a molecular structure.

The term "prodrug" as used herein intends a latent form of an active drug with certain physicochemical properties that allow it to reach a target organ or tissue. Once there, the active drug is formed chemically or enzymatically *in situ*.

The term "corticosteroid" is intended to mean not only steroids produced by the natural cortex but also synthetic equivalents, i.e., nonnaturally occurring steroids which possess physiological properties characteristic of naturally occurring corticosteroids. Typical corticosteroids useful in connection with the present invention include those set forth in U.S. Patent No. 4,469,689 to Anderson et al., the disclosure of which is incorporated by reference herein. "Natural" intends compounds which are produced in nature, more particularly those produced in living organisms, while "synthetic" refers to compounds produced by chemical synthesis.

The term "sugar" as used herein is intended to mean monosaccharides and oligosaccharides containing 2-6 sugar residues. It is also intended that the term include those species in which one or more oxygen atoms in the sugar moiety have been replaced with sulfur atoms, or wherein one or more hydroxyl groups have been replaced with primary amine groups, or wherein the carbon atom bearing the primary hydroxyl group is oxidized to a carboxyl group, i.e., uronic acids.

The term "lower alkyl" as used herein in the description of chemical structures is intended to encompass alkyl groups having 1 through 6, preferably 1 through 4, carbon atoms.

The term "colon-specific" as used herein means that drug delivery is essentially exclusive to the colonic area of the mammalian gastrointestinal tract. "Colon-specific drug delivery" means that at least about 30 wt.% of the drug administered reaches the large intestine.

By the term "effective" amount of a drug is meant a nontoxic but sufficient amount of the drug, to provide the desired local effect and performance at a reasonable benefit/risk ratio attending any medical treatment.

The term "pharmaceutical carrier" as used herein refers to a carrier suitable for oral administration of a drug, and includes any such materials known in the art, e.g., any liquid or nonliquid vehicle which is stable with respect to the prodrug and any other components present in the pharmaceutical composition.

"Endogenous enzymes" as used herein are enzymes produced by the mammalian host (as opposed to enzymes produced by bacteria found within the mammalian intestine) that are secreted into the mammalian gastrointestinal tract.

The Prodrugs of the Invention:

The corticosteroid prodrugs of the invention comprise a corticosteroid bound through its 21-OH group to a sugar species, and may thus be represented as R-X-St where R represents the sugar, X is

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sulfur or oxygen, and St represents the corticosteroid. Typically, the prodrugs will fall within the following structural group represented by Formula (III)

(III)
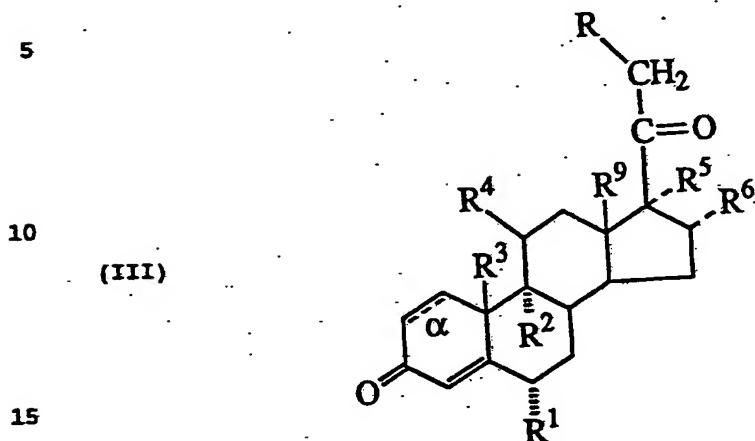
In the formula:

R, as noted above, represents a sugar moiety;

R^1 is selected from the group consisting of H, halogen and lower alkyl;

R^2 is selected from the group consisting of H and halogen;

R^3 is selected from the group consisting of H and lower alkyl;

R^4 is selected from the group consisting of OH and oxo;

R^5 is selected from the group consisting of H and OH;

R^6 is selected from the group consisting of H, lower alkyl and $-OCOR^7$ where R^7 is lower alkyl, or wherein R^5 and R^6 together form an $-O-R^8-O-$ bridge wherein R^8 is lower alkylene;

R^9 is selected from the group consisting of H and lower alkyl; and

α represents an optional double bond.

Examples of particularly preferred corticosteroids which may be administered using the present method include dexamethasone, betamethasone, beclomethasone, budesonide, prednisone, prednisolone, methyl prednisolone, flunisolide, triamcinolone acetonide, flucinolone acetonide, flumethasone, chlorprednisone, fluprednisolone, 11-deoxycorticosterone, 9α-fluorohydrocortisone, paramethasone and dehydrocorticosterone.

The sugar moiety R should be selected so that it enables gradual hydrolysis of the prodrug, such that the corticosteroid can be delivered to the selected site within the colon. Preferred

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sugars are those which are hydrolyzed by glycosidases present in the caecum and colon at rates less than about 300 nmol/h/mg protein as measured using the procedure described by G.T. MacFarlane et al. in Letters in Applied Microbiology 12:3-7 (1991), the disclosure of which is incorporated by reference herein. This group includes chitobide (hydrolyzed by chitobiase), α- and β-mannose (hydrolyzed by α- and β-mannosidase, respectively), and uronic acids. Where R is a uronic acid, it will typically have a structure selected from the group consisting of
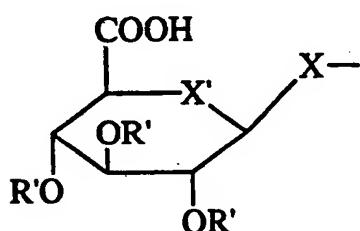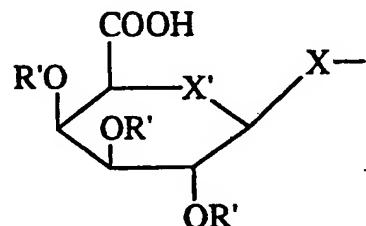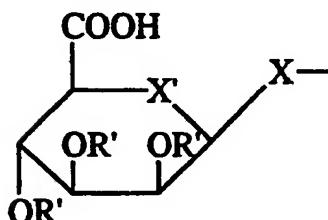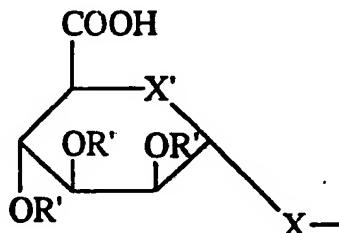
where X is oxygen or sulfur, X', similarly, is either oxygen or sulfur, and R' is selected from the group consisting of hydrogen and lower alkyl. β-D-glucuronic acid and β-galacturonic acid (hydrolyzed by β-D-glucuronidase and β-D-galacturonidase, respectively), are particularly preferred.

Synthesis of the Prodrugs:
To form a corticosteroid-glucuronide prodrug to be utilized in the present colon-specific drug delivery system, a sugar residue is attached to the drug aglycone to create a synthetic drug glucuronide. These drug glucuronides can be synthesized using known chemical techniques. See Igarashi, K., Adv. Carbohydr. Chem. Biochem. 34:243-283 (1977). An especially preferred method is the Koenigs-Knorr reaction. See Meystre, C., and Miescher, K., Helv. Chim. Acta. 28:1153-1160 (1944); Koenigs, W., and Knorr, E., Ber. 34:957-981 (1901); and Igarashi, K., Adv. Carbohydr. Chem. Biochem. 34:243-283 (1977). This reaction involves the conjugation of a bromosugar derivative to the C-21 hydroxyl group of the selected

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corticosteroid, and may also be used to synthesize prodrugs using
other sugar moieties, e.g., chitobide, α- or β-mannose, and the
like. The product is isolated by column chromatography and then
deacylated and demethylated in the presence of dilute sodium
hydroxide.

5   If the linkage between the corticosteroid and the sugar
moiety is to be a thioether, such that "X" is sulfur, the
corticosteroid must be modified prior to conjugation to the sugar.
Such modification may be carried out using techniques well-known to
those skilled in the art of synthetic organic chemistry. One such
10   method involves conversion of the C-21 hydroxyl functionality to a
halogen, typically chloro or bromo, followed by reaction with
hydrogen sulfide or the bisulfide ion.

         Utility and Administration:
15   The prodrug is preferably administered orally to the
mammalian host. The prodrug is then allowed to pass through the
mammalian host's gastrointestinal system. Since the synthetic
prodrug is larger and more hydrophilic than the parent drug, the
prodrug is less permeable than the parent drug. In addition, since
20   the glycosidic bond linking the uronic acid moiety to the steroid is
a bond that will be substantially selectively cleaved by enzymes
produced by colonic microflora, the synthetic prodrug will pass
through the gastrointestinal tract without being significantly
absorbed from the gastrointestinal tract or without being
25   significantly hydrolyzed by endogenous enzymes produced by the
mammalian host. Once in the area of the colon, the prodrug will be
acted upon by bacterial glycosidases, thus releasing free drug for
adsorption to or absorption by the colonic mucosa.
         For oral administration, a pharmaceutically acceptable
30   nontoxic composition is formed by the incorporation of any of the
normally employed excipients, such as, for example, pharmaceutical
grades of mannitol, lactose, starch, magnesium stearate, sodium
saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate
and the like. Such compositions take the form of solutions,
35   suspensions, tablets, pills, capsules, powders, sustained release
formulations and the like. Such compositions will typically contain
10% to 95% prodrug. Preferred pharmaceutical compositions are
solutions or suspensions, e.g., in water, saline, aqueous dextrose,
glycerol, ethanol, or the like; a particularly preferred composition
is a simple sterile aqueous solution. The composition may contain,
in addition to prodrug and carrier, auxiliary substances such as
emulsifiers, pH buffering agents, and the like. Actual methods of
preparing such compositions are known, or will be apparent to those

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skilled in the art; for example, see Remington's Pharmaceutical
Sciences, Mack Publishing Company, Easton PA.

      The amount of prodrug administered will, of course, be
dependent on the subject being treated, the severity of the
affliction, the manner of administration and the judgment of the
prescribing physician. The dosage of prodrug will be significantly
lower relative to the required dosage for the corresponding drug.
For example, an effective dosage of prednisone is in the range of
approximately 0.5 to 1.0 mg/kg/day, preferably about 0.5 mg/kg/day.
For an average 70 kg human, this would amount to approximately 35 mg
per day. For dexamethasone, an effective dosage is typically in the
range of 0.08 to 0.16 mg/kg/day, preferably about 0.8 mg/kg/day
(again, for an average 70 kg human, this amounts to about 5.6 mg per
day). For the corresponding prodrugs, required dosage to achieve
the same results will be reduced by at least a factor of ten. The
dosage will generally be about the same (i.e., equimolar) for the
different prodrugs of the invention, formed from varying
corticosteroids and sugar moieties.

      The present prodrugs of the invention have been
demonstrated to be highly effective in the colon-specific delivery
of corticosteroids with a minimum of systemic side effects. While
not wishing to be bound by theory, the fact that the present
prodrugs are particularly suited for very specific colonic delivery
is believed to be due at least in part to the fact that the prodrugs
exhibit extensive first-pass metabolism in the colon and liver. The
new prodrugs have been found to provide a significant "selective
advantage" relative to previously known methods of administering
corticosteroid drugs.

      It is to be understood that while the invention has been
described in conjunction with preferred specific embodiments
thereof, the foregoing description, as well as the examples which
follow, are intended to illustrate and not limit the scope of the
invention. Other aspects, advantages and modifications within the
scope of the invention will be apparent to those skilled in the art
to which the invention pertains.

Experimental

      Solvents: All solvents were redistilled and dried over
molecular sieves, 4 angstrom, 4-8 mesh (Aldrich Chemical Co.). All
solvent evaporations were performed with a rotary evaporator with
water aspirator reduced pressure. Melting points were obtained on a
Buchi melting point apparatus and are uncorrected. UV spectra were

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determined on a Cary 210 spectrometer. IR spectra were determined
on a Perkin Elmer Model 137 spectrometer. ^1H NMR spectra were
determined on a Gemini 300 MHz device at SRI International, Menlo
Park, CA, and were recorded in dimethyl-d_6 sulfoxide; they are
expressed in parts per million (delta) downfield from Me$_4$Si with
5   coupling constants (J) expressed in hertz.

Chromatography: High-pressure liquid chromatography
(HPLC) was performed on a Waters 840 system consisting of two model
510 pumps, a model 481 UV detector, a model 710B WISP (automatic
sampler), and a Digital Computer Model 350 (4.6 x 25 cm, 5
10   micrometer Ultrasphere C-18). A flow rate of 1.2 mL/min was used,
with absorbance monitoring at 254 nm. The solvent system for all
separations was MeOH/0.01 M KH$_2$PO$_4$ (56.5:43.5). Low-pressure
preparative chromatography (flash chromatography, J.T. Baker Chem.
Co.) was performed using either a 3.7 x 22 cm column of 40
15   micrometer RP-18 with MeOH/water (68:32) as eluent or MeOH/water
(50:50) as eluent. TLC was performed on aluminum-backed plates of
silica gel 60 (E. Merck Co.). Steroids and their glycosides were
identified using a UV lamp.

20   Example 1
                    Dexamethasone Glucuronide Prodrugs:
                       Synthesis and Characterization
             (a.) Preparation of 9α-fluoro-11β,17α-
       dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl methyl 2,3,4-
25   tri-O-acetyl-β-D-glucosiduronate: Dexamethasone-methyl acetyl
glucuronate was prepared by dissolving dexamethasone (2.2 g, 5.6
mmol) in chloroform (300 mL) and heating to reflux in a 500 mL round
bottom flask over 4-Å molecular sieves. After approximately 10 mL
had been distilled over freshly prepared silver carbonate (8.6 g,
30   31.7 mmol) was added to the flask, followed by the dropwise addition
of methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)-uronate (5.3 g,
14.1 mmol; Sigma Chemical Co., St. Louis, MO) in chloroform (150 mL)
over a 1 h period. The reaction vessel was protected from light
during the addition of bromosugar. The reaction mixture was
35   refluxed an additional 2 h after which time the reaction mixture was
filtered and the solution washed with cold saturated NaCl solution,
and dried (Na$_2$SO$_4$) and the solvent removed. The oily residue was
dissolved in several mL of MeOH and purified by flash chromatography
on 40 μm reversed phase C-18 (eluent: MeOH/water, 65:35, v/v). The
appropriate fractions were collected, and the solvent was removed.
Dexamethasone methyl acetyl glucuronide was crystallized from
MeOH/water to yield 980 mg (24.5%): mp 135-137°C; IR (KBr) 3450
(OH), 1755 (OAc), 1660 (C=O), 1620 (C=C), 1220 (OAc), 1040, 890, 690
cm^{-1}; ^1H NMR δ 0.87 (s, 3 H, C-18), 1.49 (s, 3 H, C-19), 1.98 (s, C-4'

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OAc), 2.00 (s, 3 H, 3' OAc), 2.04 (s, 3 H, 2' OAc), 4.28 (d, 1 H, C-1', J=7.2 Hz), 4.49 (AB q, 2 H, C-21, J=18.3 Hz), 6.00 (d, 1 H, C-2, J=7.2 Hz), 7.28 (d, 1 H, C-1, J=18.3 Hz).

(b.) Preparation of 9α-Fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl β-D-glucosiduronate:

5   Dexamethasone-β-D-glucuronide was prepared according to the procedure of Mattox, et al., Biochemistry 8:1188 (1969). Dexamethasone-methyl acetyl glucuronate (500 mg, 0.7 mmol) was dissolved in 0.2 N methanolic sodium hydroxide (50 mL) until homogeneous (about 5 min). After an additional 5 min, water (50 mL)

10 was added and the reaction solution was stirred for 5 additional min. Then, dilute acetic acid in methanol was added to neutralize the basic solution to pH 7.5. The solvent was removed under reduced pressure and the residue was dissolved in about 2-3 mL of H₂O/MeOH (50:50, v/v) and then purified by flash chromatography on reversed

15 phase C-18 (H₂O/MeOH, 50:50, v/v). The appropriate fractions were collected and the solvent was removed under reduced pressure. The residue was dissolved in pure water (20-30 mL) and the product collected by lyophilization to give 365 mg (91%) of dexamethasone-β-D-glucuronide (sodium salt): IR (KBr) 3450 (OH), 1720 (C=O), 1660

20 (C=C), 1600 (COO⁻); ¹H NMR δ 0.87 (s, 3 H, C-18), 3.31 (s, 5 H, OH), 4.28 (d, 1 H, C-1', J=7.2 Hz), 4.49 (AB q, 2 H, C-21, J=18.3 Hz), 6.00 (d, 1 H, C-2, J=7.2 Hz), 7.28 (d, 1 H, C-1, J=18.3 Hz).

Octanol/Water Partition Coefficient of Dexamethasone-β-D-glucuronide: The partitioning of dexamethasone-β-D-glucuronide

25 was measured between 1-octanol and an aqueous phase (0.05 M sodium phosphate buffer, pH 7.4) at room temperature. Both octanol and buffer were saturated with the relevant aqueous or organic phase before use. Equal volumes (3.0 mL) of both phase were used and agitated overnight. The initial concentration of glucuronide was

30 0.1 mg/mL, dissolved in the buffer. The amount of glucuronide in the aqueous phase at equilibrium was measured spectrophotometrically at 242 nm. Concentration in 1-octanol was determined by difference.

Example 2

35 Budesonide Glucuronide Prodrugs:
Synthesis and Characterization

(a.) Preparation of budesonide-21-yl methyl 2,3,4-tri-O-acetyl-β-D-glucosiduronate: Budesonide-methyl acetyl glucuronate was prepared by dissolving budesonide (650 mg, 1.5 mmol) in chloroform (200 mL) and heating to reflux in a 500 mL round-bottom flask over 4-Å molecular sieves. After approximately 10 mL had been distilled over, freshly prepared silver carbonate 3.0 g, 11 mmol) was added to the flask followed by the dropwise addition of methyl(tri-O-acetyl-α-D-glucopyranosyl bromide)-uronate (2.2 g, 5.9

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mmol; Sigma Chemical Co.) in chloroform (100 mL) over a 1 hr period.
The reaction vessel was protected from light during the addition of
bromosugar. The reaction mixture was refluxed an additional 1 hr
after which time the reaction mixture was filtered and the solution
washed with cold saturated NaCl solution, and dried (Na$_2$SO$_4$) and the

5 solvent removed. The oily residue was dissolved in several
milliliters of MeOH and purified by flash chromatography on 40 μm
reversed phase C-18 (eluent:MeOH/water, 65:35, v/v). The
appropriate fractions were collected, and the solvent was removed.
Budesonide methyl acetyl glucuronide was crystallized from

10 MeOH/water to yield 240 mg (19%):mp 135-137°C; IR (KBr) 3450 (OH),
1755 (OAc), 1665 (C=O), 1620 (C=C), 1220 (OAc), 1035, 890, 690 cm^{-1};
^{1}H NMR δ 0.87 (s, 3 H, C-18), 1.49 (s, 3 H, C-19), 1.98 (s, C-4'
OAc), 2.00 (s, 3 H, 3' OAc), 2.04 (s, 3 H, 2' OAc), 4.28 (d, 1 H, C-
1', J=7.2 Hz), 4.49 (AB q, 2 H, C-21, J=18.2 Hz), 6.00 (d, 1 H, C-2,

15 J=7.2 Hz), 7.18 (d, 1 H, C-1, J=18.4 Hz).
         (b.) Preparation of budesonide-21-yl
β-D-glucosiduronate: Budesonide-β-D-glucuronide was prepared
according to the procedure of Mattox et al., Biochemistry 8:1188
(1969). Budesonide-methyl acetyl glucuronate (200 mg, 0.7 mmol) was

20 dissolved in 0.1 N methanolic sodium hydroxide (50 mL) until
homogeneous (about 5 min). After an additional 30 min, water (50
mL) was added and the reaction solution was stirred for 30
additional min. Then dilute acetic acid in methanol was added to
neutralize the basic solution to pH 7.5. The solvent was removed

25 under reduced pressure and the residue was dissolved in about 2-3 mL
of H$_2$O/MeOH (50:50, v/v) and then purified by flash chromatography
on reversed phase C-18 (H$_2$O/MeOH, 50:50, v/v). The appropriate
fractions were collected and the solvent was removed under reduced
pressure. The residue was dissolved in pure water (20-30 mL) and

30 the product collected by lyophilization to give 130 mg (81%) of
budesonide-β-D-glucuronide (sodium salt):IR (KBr) 3450 (OH), 1720
(C=O), 1660 (C=C), 1600 (COO$^-$); ^{1}H NMR δ 0.87 (s, 3 H, C-18), 3.31
(s, 5 H, OH), 4.28 (d, 1 H, C-1', J=7.4 Hz), 4.49 (AB q, 2 H, C-21,
J=18.2 Hz), 6.00 (d, 1 H, C-2, J=7.2 Hz), 7.29 (d, 1 H, C-1, J=18.3

35 Hz).

Example 3

Prodrug Evaluation: Dexamethasone-β-D-glucuronide

         (a.) Hydrolysis of model substrates: A number of
experiments were performed on the male Sprague Dawley rat (200-250
g). The activity of β-D-glucosidase and β-D-glucuronidase was
measured in luminal contents, intestinal mucosal scrapings, and the
remaining muscle layers of the gastrointestinal tract (GIT) of
conventional rats using p-nitrophenyl substrates. In addition, the

same experiments were repeated in germ-free (GF) rats and in rats with experimental colitis. The data collected in the *in vitro* hydrolysis experiments are presented in Figures 1 through 3. The glucuronidase activity was measured at two pH values: 4.5 and 6.5.
5   This is because bacterial β-D-glucuronidase activity has a pH optimum close to 6.5 while lysosomal β-D-glucuronidase activity (endogenous enzyme) has a pH optimum close to 4.5. The results were normalized to total protein content.

As expected, the highest hydrolytic activity was observed in the cecal and colonic contents, whereas the contents of
10   stomach, PSI, and DSI had much lower activity (see Figure 1A). The PSI has a significantly higher level of β-D-glucosidase activity compared with β-D-glucuronidase activity (p<0.01, ANOVA), while levels were comparable in the DSI. There was a 30-fold increase in specific activity of luminal β-D-gluronidase activity between the
15   DSI and cecum in the conventional rats; the gradient of activity of β-D-glucosidase between the DSI and cecum was less pronounced. The GF rats showed a much lower level of β-D-glucosidase activity and β-D-glucuronidase activity in the cecum and colon compared with conventional rats (see Figure 1B). There was also a higher level of
20   β-D-glucosidase activity in lumen of the PSI and DSI compared with luminal β-D-glucuronidase activity. While not wishing to be bound by theory, this activity is believed to be due to sloughed mucosal cells. Figure 1C shows the same general pattern of glycosidase activity in the acetic acid-induced colitis model as observed in the
25   conventional rats. Luminal β-D-glucosidase activity (endogenous enzyme) was slightly higher in the PSI than in the DSI. In the cecum and colon, there was no difference statistically between luminal activity of conventional and colitis-induced rats. This relationship was also found with luminal β-D-glucuronidase activity
30   in the PSI and DSI; in contrast, there was a statistically significant difference between the activities in the cecum (p<0.05) and colon (p<0.01).

Figure 2 shows the activities of β-D-glucosidase and β-D-glucuronidase in mucosal scrapings from conventional, GF, and
35   colitis-induced rats. Mucosal β-D-glucosidase activity was measured at pH 6.5; β-D-glucuronidase activity was measured at pH 6.5 and 4.5. Lysosomal β-D-glucuronidase activity is greater at pH 4.5 than at pH 6.5, which is close to the optimum for bacterial β-D-glucuronidase (Hsu, L., and Tappel, A.L., Biochim. Biophys. Acta 101:83-89 (1965); Hsu, L., and Tappel, A.L., J. Cell Biol. 23:233-240 (1964)). It was found that mucosal β-D-glucuronidase activity was greater in the large intestine than in the small intestine (see Figure 2A), a finding consistent with Hsu and Tappel (supra). Mucosal β-D-glucuronidase activity was significantly higher at pH

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4.5 than at pH 6.5 in the mucosal scrapings. The GF rats showed
significantly (ANOVA, Bonferroni t-test, p<0.002) PSI and DSI less
β-D-glucuronidase activity at pH 4.5 than both conventional and
colitic rats. In the colitis-induced rats, mucosal glycosidase
activity varied only slightly along the entire length of the rat
GIT. Mucosal scrapings from the cecum and colon of the colitis-
induced rats showed qualitatively lower activity than the
conventional rats at pH 4.5.

Homogenates of the gastrointestinal muscle tissues (see
Figure 3) showed lower levels of β-D-glucosidase than the
corresponding mucosal layers. Levels in the conventional GF, and
colitis-induced rats were highest in the PSI compared with the rest
of the GIT. In the case of β-D-glucuronidase activity at pH 4.5,
there was a steady decrease in activity from PSI to the cecum.
There was no significant difference of muscle tissue β-D-
glucuronidase levels between the conventional, GF, and colitis-
induced rats at any site examined along the GIT. Apparently,
neither a lack of gut microflora nor induction of colitis with
acetic acid affects the β-D-glucosidase and β-D-glucuronidase
activities of the GIT muscle layers.

Overall, the sharper difference in luminal
β-D-glucuronidase activity between the DSI and cecum compared with
luminal β-D-glucosidase activity suggests that β-D-glucuronides
would be less susceptible to premature hydrolysis in the DSI than β-
D-glucosides. The higher level of β-D-glucosidase activity in the
lumen of the PSI compared with the DSI may be due to sloughed
mucosal cells. Data from the GF rats supports this hypothesis.
This data suggests that the glucoside prodrugs would not be as
stable in the PSI and hence would be less suitable for colonic
delivery than glucuronide prodrugs.

β-D-glucuronidase activity in the mucosa and muscle
layers appears to be lysosomal in origin based on pH activity data.
To effect drug delivery, the glucuronide prodrugs would need to
enter not only the cell, but the lysosomal compartment, in order to
be hydrolyzed in vivo. In addition, β-D-glucuronidase activity from
lysed exfoliated mucosal cells will be reduced in the less acidic
luminal contents. Therefore, glucuronide prodrugs should be more
stable in the PSI and hence more suitable than glucoside prodrugs.

(b.) Hydrolysis of Glycoside Prodrugs: The hydrolysis
of a glucuronide prodrug was also examined in vitro using the rat.
The prodrug dexamethasone-β-D-glucuronide (synthesized in Example 1)
was incubated with the intestinal contents, mucosal scrapings, and
the remaining muscle tissue.

The luminal contents of fed rats were removed following
sacrifice by flushing with chilled 0.9% NaCl. The contents were

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then homogenized (Ultra Turrax) for 2 min with cooling on ice. The
PSI and DSI contents were diluted to 20-25 g with 0.9% NaCl while
the cecal and colonic contents were diluted to 40-45 g with 0.9%
NaCl. The mucosal scrapings were diluted with 6-10 with 0.9% NaCl.
Homogenization (Ultra Turrax) was performed for 30 s (ice cooling).
5   Homogenates of tissues (muscle layer) was performed by first cutting
the tissues into small pieces and diluting to 20 g with 0.9% NaCl.
The tissues were then homogenized for 30 s with a Polytron
Homogenizer. All homogenates were centrifuged for 10 min at 1,500
g. The supernatants were used for hydrolysis experiments followed
10   by determination of total protein using the Lowry technique.

The substrate (dexamethasone-β-D-glucuronide)
concentration used was 0.5 mM. This concentration is K_m (determined
in a preliminary experiment). The pH of the incubation mixtures was
adjusted to 6.5 and 4.5 with 0.1 M sodium phosphate buffer and 0.1 M
15   sodium acetate buffer, respectively. The reaction was started by
adding the substrate at 37°C (total volume=2.5 mL; triplicate). At
t=0 and t=60 min, 0.5 mL aliquots were withdrawn and the reaction
stopped by adding the aliquot to 1.0 mL saturated NaCl (ice cold),
which contained the 25 μL of the internal standard solution
20   (equilenine for contents and triamcinolone acetonide for mucosa and
tissues). The stopped reactions were extracted twice with methyl
t-butyl ether/pentane (6:4, v/v) by vortexing for 5-10 s followed by
centrifugation (3,500 g, 5 min). The organic phase was collected
and evaporated to dryness under argon (Meyers-N_2-Evaporator). The
25   residue was reconstituted in 120 μL of water/acetonitrile (1:1,
v/v). After spinning on an Eppendorf microfuge, 80 μL was injected
onto the HPLC.

For the HPLC, a reversed phase system was used
consisting of Whatman Partisil ODS-3, 10 μm, 3.9 x 300 mm. The
30   mobile phase was 0.02 M acetate buffer, pH 4.8/acetonitrile (68:32).
The flow rate was 1 mL/min (room temperature) and the detector was
set at 246 nm. The Waters 840 chromatography system consisted of
two model 510 pumps, a model 481 UV detector, a model 710B WISP
(sample processor), and a Digital Computer model 350.
35   The data collected in these experiments are shown in
Figure 4. As shown in Figure 4A, there was an 80-fold increase in
activity between the DSI and cecum. Compared with the hydrolysis of
p-nitrophenyl-β-D-glucuronide, the rate of hydrolysis of the prodrug
is about 10-50-fold lower in the cecum/colon contents. The
hydrolysis of dexamethasone-β-D-glucuronide in the mucosal scrapings
along the rat GIT (see Figure 4B) was much lower than that observed
in the contents. From the relatively high level of activity in the
cecal mucosa at pH 6.5, it appears that the contents may not have
been completely removed from the mucosa. The hydrolysis data at pH

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4.5 provides a better measure of mucosal activity derived from
lysosomes. As expected, the hydrolysis of the prodrug in the muscle
tissues is very low (see Figure 4C).

The *in vitro* hydrolysis of dexamethasone-β-D-glucoside
in guinea pigs was similarly evaluated (see T.N. Tozer et al.,
5   Pharmaceutical Research 8(4):445-454 (1991), the disclosure of which
is incorporated by reference herein). The greatest hydrolytic
activity was found in the luminal contents of the cecum and colon;
contents of the stomach, PSI, and distal small intestine (DSI) had
much lower activity. In contract, activity in the tissues was
10   greatest in the PSI and DSI with little activity in the tissues of
the stomach, cecum, and colon. Similar results were obtained with
dexamethasone-β-D-cellobioside; there was very little hydrolysis in
the contents of the stomach, DSI, and PSI with much higher relative
rates in the large intestine.

15   (c.) *In Vivo* Studies: Pharmacokinetics of Drug
Delivery: In previous studies, it was found that both dexamethasone
and prednisolone could be carried to the rat large intestine using
glucoside prodrugs and that the active agent was released there.
(Friend, D.R., and Chang, G.W., J. Med. Chem. 27:261-266 (1984).
20   Delivery of dexamethasone, using the prodrug dexamethasoneβ-D-
glucoside, to the guinea pig large intestine, has also been
demonstrated (see Tozer et al., *supra*).

(d.) *In vitro* distribution studies in the guinea pig:
The following studies were performed to identify where in the guinea
25   pig GIT the prodrug was being hydrolyzed and, once hydrolysis
occurred, whether the compound was absorbed by the intestinal
tissues. Dexamethasone was also administered intravenously (i.v.)
to guinea pigs and the plasma and cecal/colonic tissue levels of
dexamethasone were measured. The data from these two experiments
30   were compared using the relationship called "selective advantage."

About 20% to 30% of an oral dose of dexamethasone-β-D-
glucoside was found to reach the large intestine and was
subsequently hydrolyzed there (no prodrug was ever recovered from
the large intestinal contents). From i.v. administration of the
35   prodrug and drug, it was apparent that the prodrug is poorly
absorbed in the GIT (bioavailability < 1%), but that significant
amounts are hydrolyzed in the small intestine. As discussed below,
humans have a much lower level of bacterial glycosidase activity in
the GIT than the guinea pig. Specific delivery is therefore
anticipated to be greater in humans than in the animal model.
Selective advantage of about 9 in the cecal tissues of the guinea
pig was calculated. Assuming linearity of pharmacologic response in
the tissues of the large intestine, the dose of dexamethasone could
be lowered nine times relative to a systemic dose without reducing

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efficacy. Because the primary problem with corticosteroids is their
side effects, such a reduction in total body exposure would be a
clear advantage in controlling IBD.

In addition to dexamethasone-β-D-glucoside, a
distribution and recovery experiment with the prodrug dexamethasone-
5   β-D-cellobioside was also performed. The distribution of
dexamethasone-β-D-cellobioside and dexamethasone in the luminal
contents and tissues after oral administration of dexamethasone-β-D-
cellobioside was similar to those obtained using dexamethasone-β-D-
glucoside. The results indicated that the cellobioside prodrug
10   delivered a significant amount of dexamethasone to the lumen of the
cecum and, to a lesser extent, the colon. This result led to
elevated tissue levels of dexamethasone. However, the overall
recovery of dexamethasone was relatively low: only 33% of the total
dose was recovered in the guinea pig GIT after 1 h; by 3 h, the
15   total recovery was about half that--15%. The cellobioside (1)
showed a low total recovery of administered dose, even at early time
points, (2) had a relatively high water solubility, and (3) had a
slower rate of hydrolysis in the intestinal contents. These
observations suggest that reduced specificity of delivery to the
20   large intestine is probably due to hydrolysis by intestinal
(mammalian) glucosidases. A similar distribution and recovery
experiment with a dexamethasone glucuronide prodrug (e.g., as
synthesized in Example 1) is expected to give rise to enhanced
specificity of delivery relative to that obtained with the
25   β-D-cellobioside and β-D-glucoside prodrugs.

(e.) *In Vivo* Studies--Efficacy: The efficacy of two
prodrugs in two different animal colitis models was addressed as
follows. Dexamethasone-β-D-glucoside and dexamethasone-β-D-
glucuronide have been studied in the carrageenan-induced colitis
30   guinea pig model and dexamethasone-β-D-glucuronide has been studied
in the acetic-acid colitis rat model. The overall goals of the
efficacy studies were to evaluate the relative efficacy of drugs and
the corresponding prodrugs.

Efficacy of dexamethasone-β-D-glucoside in treating
35   carrageenan-induced colitis in guinea pigs: Two studies were
performed using guinea pigs with experimentally induced IBD. IBD
was induced by 4-5 wt.% degraded carrageenan in the drinking water
for 14 days; on day 15, the animals were dosed with an equivalent of
0.5 mg/kg or 0.25 mg/kg of dexamethasone-β-D-glucoside,
dexamethasone, or with the dosing vehicle only, once daily for 5
days. The efficacy of the prodrug and drug was assessed by the
number of ulcers in control and treated guinea pigs. Relative to
control animals, the drug and prodrug treatments were found to
result in significantly fewer ulcers. There was no difference

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statistically between the drug and prodrug groups at the dose examined.

Efficacy of dexamethasone-β-D-glucuronide in treating carrageenan-induced colitis in guinea pigs: Substantially the same procedure was repeated using dexamethasone-β-D-glucuronide. The
5   animals were divided into three groups that received the following treatments from day 15 to 20: 1--H_2O/EtOH (95:5, v/v); 2-- dexamethasone (1.3 μmol/kg); and 3--dexamethasone-β-D-glucuronide (1.3 μmol/kg). These treatments were administered once daily in 1 mL of the H_2O/EtOH dosing vehicle. Results are summarized
10   graphically in Figure 5, with the mean number of ulcers plotted for each treatment group. Using ANOVA followed by the Bonferroni t-test for multiple comparisons, it was found that the reduction in ulcers was significantly different (p < 0.05) in the animals treated with dexamethasone-β-D-glucuronide compared with control animals, while
15   that obtained from only dexamethasone was insignificantly different. No ulcers were detected in animals treated only with the dosing vehicle (no carrageenan treatment). Results were clearly better than those obtained with the glucoside prodrug of the preceding experiment.
20   Efficacy of Dexamethasone-β-D-glucuronide in treating acetic acid-induced colitis in rats:

The prodrug dexamethasone-β-D-glucuronide was tested in the acetic acid-induced colitis rat model, as follows. Acetic acid colitis was induced as described by Fedorak et al., Gastroenterology
25   98:615-625 (1990) (incorporated by reference herein). Following induction of colitis in the rats (n=5-8 for each group), the animals were administered dexamethasone or dexamethasone-β-D-glucuronide at levels of 0.44, 0.22, 0.055, or 0.0137 μmol/kg/d orally (the typical human dose of dexamethasone is 0.22 μmol/kg/d for systemic treatment
30   of colitis) at 24 and 48 h (once daily at days 1 and 2 following induction of colitis). The in vivo colonic fluid absorption was measured 72 h after induction of colitis. Fluid absorption measurements in vivo provide a very sensitive measure of tissue damage. In vivo fluid flow abnormalities can persist even when
35   morphometric, histologic and other indicators of inflammation have returned to normal (Fedorak et al., Gastroenterology 98:615-625 (1990)). Decreased water absorption is a common clinical feature in both ulcerative colitis and Crohn's disease (Phillips, in Inflammatory Bowel Disease, Kirsner & Shorter, Eds., Lea & Febiger, Philadelphia, 1988; pp.239-256). The absorption/secretion data of treatment and control groups are shown in Figure 5. There was substantial improvement in all the prodrug treated animals. At lower doses, it was apparent that dexamethasone was ineffective (0.0137 μmol/kg/d) compared with control animals (4% AAC) whereas

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dexamethasone-β-D-glucuronide at 0.0137 μmol/kg/d returned fluid flow to net absorption. All treatment groups except the low dose dexamethasone (0.0137 μmol/kg/d) significantly improved fluid absorption compared with the acetic acid control animals (Bonferonni t-test, $p<0.05$). Comparing the low dose drug with the low dose prodrug (0.0137 μmol/kg/d), the prodrug significantly improved colonic fluid flow relative to the drug (Student-Newman Keuls t-test $p<0.05$).

The colonic fluid flow data can be expressed in a different manner to obtain a relative effectiveness of each agent in 50% of the animals (ED_{50}). To do this, a nonlinear regression program *Minim* (Version 1.8a, Dr. R. Purves, Department of Pharmacology, University of Otago, Dunedin, New Zealand) was used to fit the Hill equation to the colonic water-absorption data. To incorporate both positive and negative values (fluid absorption and secretion values, respectively, the equation was modified to include the term $Effect_0$ (μl/h/cm), as follows:

$$Effect = Effect_0 + \frac{(Effect_{max})(Dose^\gamma)}{(ED_{50} + Dose^\gamma)}$$

where:

Effect	=	Water flux (μl/h/cm of intestine)	
$Effect_0$	=	Water flux in animals with acetic acid-induced colitis (μl/h/cm of intestine	
$Effect_{max}$	=	−Effect + Control (Control is the value in animals not treated with either 4% acetic acid or drug) (μl/h/cm of intestine)	
γ	=	Hill coefficient	
ED_{50}	=	Daily dose which give half maximal effect (μ/l/h/cm of intestine)	

Figure 6 shows the plot of fluid flow versus log dose using the Hill equation. The ED_{50} calculated for dexamethasone, based on *in vivo* fluid flow, is 1.8×10^{-1} μmol/kg/d and 1.25×10^{-2} μmol/kg/d for dexamethasone-β-D-glucuronide. Therefore, the prodrug is 16 times more effective than the parent drug dexamethasone. This means that a 16-fold lower dose, on a molar basis, of dexamethasone-β-D-glucuronide compared with dexamethasone can be used with similar antiinflammatory effect. Such a reduction in dose using the prodrug will have an effect on levels of circulating adrenocorticotropic hormone (ACTH) and corticosterone levels, both of which if lowered in mammals, leads directly to the serious complications normally

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associated with glucocorticosteroid therapy (Haynes and Murad, in: Goodman's and Gilman's The Pharmacological Basis of Therapeutics, 6th Ed., Goodman, Goodman and Gilman, Eds., Macmillan, New York, 1980, Chapter 63). Serum ACTH levels in the same animals used to

5   collect fluid flow data are shown in Table 1 for the dexamethasone and dexamethasone-β-D-glucuronide treated rats. The low dose (0.0137 μmol/kg/d) dexamethasone and dexamethasone-β-D-glucuronide did not significantly lower ACTH levels compared with the control colitis animals; however, the low dose dexamethasone was totally ineffective at controlling water secretion/absorption while the

10 prodrug at this dose level was effective. The higher dose dexamethasone and dexamethasone-β-D-glucuronide (0.44 μmol/kg/d) significantly depressed serum ACTH levels compared with the acetic acid control animals (ANOVA, Student-Newman-Keuls t-test, $p < 0.05$). The corticosterone levels in the rats were elevated somewhat in the

15 colitic animals (see Table 1). Compared with control rats, the low dose dexamethasone-β-D-glucuronide (0.0137 μmol/kg/d) did not significantly reduce serum corticosterone levels, while the equal dose of dexamethasone (0.0137 μmol/kg/d) did significantly lower serum corticosterone levels (Student-Newman-Kuels t-test, $p < 0.05$).

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Table 1
Serum ACTH (ng/L) and Corticosterone in Rats With Acid-Induced Colitis Receiving No Therapy, Dexamethasone, or Dexamethasone-β-D-glucuronide[*]

Group	Serum ACTH (ng/mL)	Cortico-sterone (μg/L)
Control (No colitis)		6.55 ± 0.22
Colitis—No therapy	1,246 ± 231	8.5 ± 0.37
Colitis + Dexamethasone (0.44 μmol/kg/d)	453 ± 158	1.82 ± 0.09
Colitis + Dexamethasone-β-D-glucuronide (0.44 μmol/kg/d)	447 ± 77	3.8 ± 0.33
Colitis + Dexamethasone (0.22 μmol/kg/d)	NP[b]	3.25 ± 0.19
Colitis + Dexamethasone-β-D-glucuronide (0.22 μmol/kg/d)	NP	4.57 ± 0.81
Colitis + Dexamethasone (0.137 μmol/kg/d)	760 ± 150	4.76 ± 0.29
Colitis + Dexamethasone-β-D-glucuronide (0.137 μmol/kg/d)	1,000 ± 178	5.68 ± 0.34

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[*] ACTH and corticosterone measurements were performed by the Endocrinology Laboratory of the University of Alberta, Edmonton, Canada. ACTH was measured using a commercial monoclonal antibody kit (Nichols Institute, San Juan Capistrano, CA). Corticosterone was measured using a commercial radioimmunoassay system from Endocrine Sciences RIA Reagents, (Tarzana, CA). Data expressed as mean ± S.E. (n=5-8).
[b] NP[b] = Not performed

In addition to measuring fluid flow in the treated acetic acid-induced colitis rats, the surface area of ulceration was measured macroscopically according to the procedure of Fedorak et al., Gastroenterology 98:615-625 (1990). Following removal of the colon from the rats at the time of sacrifice, the colon was opened longitudinally and placed flat, mucosal side upwards, on a glass plate chilled at 4°C. A transparent acetate was placed 5 mm above the mucosal surface and the area of ulceration and total surface area were traced by a single observer. Areas in square centimeters were then calculated using a Zeiss computerized videoscope (Videoplan, Carl Zeiss Co., Toronto, Ontario, Canada). This type of measurement addresses directly the ability of a drug therapy to reduce and control ulceration in the colon. The ulcerated surface area measurements for the various groups treated with dexamethasone and dexamethasone-β-D-glucuronide are shown in Figure 7. The 0.0137 μmol/kg/d dose of dexamethasone-β-D-glucuronide significantly reduced the area of ulceration compared with control AAC animals,

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while the equal dose of dexamethasone did not (Student-Newman-Kuels t-test, p<0.05).

Example 4

Prodrug Evaluation: Budesonide-β-D-Glucuronide

The prodrug budesonide-β-D-glucuronide was evaluated against budesonide in a manner similar to that used to evaluate dexamethasone and its glucuronide prodrug as described in Example 3. Fluid flow data collected is shown in Figure 8. The colonic fluid flow data (Figure 8) was evaluated statistically. The results indicate that the prodrug is more effective than the drug at each dose examined (Student-Newman-Kulls t-test, p< 0.05). The Bonferroni t-test indicates that the low dose of prodrug (0.0137 μmol/kg/d) significantly improved colonic fluid relative to the control acetic-acid rats while the low dose drug (0.0137 μmol/kg/d) did not significantly improve colonic fluid flow. Surface area of ulceration in the various groups is shown in Figure 9, while Table 2 shows corticosterone serum levels in the same animals. Surface area measurements of ulceration (Figure 9) were evaluated and it was found that the low dose prodrug (0.0137 μmol/kg/d) significantly lowered the area of ulceration while the low dose budesonide (0.0137 μmol/kg/d) did not (ANOVA, Student-Newman-Kulls t-test, p <0.05). For the corticosterone data collected (Table 2), statistical analysis indicated that only the prodrug, at all doses examined, did not significantly lower serum corticosterone levels compared with the acetic acid controls. In contrast, all the budesonide doses significantly lowered serum corticosterone levels, compared with the ACC control animals (ANOVA, Bonferonni t-test, p 0.05).

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Table 2
Serum ACTH (ng/L) and Corticosterone in Rats With Acid-Induced Colitis Receiving No Therapy, Budesonide, or Budesonide-β-D-glucuronide*

Group	Serum Corticosterone (μg/L)
Colitis—No therapy	7.0 ± 0.27
Colitis + Budesonide (0.44 μmol/kg/d)	3.9 ± 0.52
Colitis + Budesonide—β-D-glucuronide (0.44 μmol/kg/d)	7.3 ± 0.15
Colitis + Budesonide (0.11 μmol/kg/d)	5.0 ± 0.21
Colitis + Budesonide—β-D-glucuronide (0.11 μmol/kg/d)	5.7 ± 1.21
Colitis + Budesonide—β-D- (0.0137 μmol/kg/d)	5.8 ± 0.31
Colitis + Budesonide—β-D-glucuronide (0.0137 μmol/kg/d)	6.6 ± 0.47

* Corticosterone measurements were performed by the Endocrinology Laboratory of the University of Alberta, Edmonton, Canada. Corticosterone was measured as in Table 1. Data expressed as mean ± S.E. (n=5-8).

Example 5
Dexamethasone Galacturonide Prodrugs

(a.) Preparation of 9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl methyl 2,3,4-tri-O-acetyl-β-D-galactosiduronate: Dexamethasone-methyl acetyl galacturonide was prepared using the method described in Example 1(a), but substituting methyl (tri-O-acetyl-α-D-galactopyranosyl bromide)-uronate for methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)-uranate. The product may be isolated in the same way as described for the product of Example 1, part (a).

(b.) Preparation of 9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl methyl β-D-galactosiduronate: Dexamethasone-methyl acetyl galacturonate is treated as described in Example 1, part (b.), with respect to dexamethasone-methyl acetyl glucuronate, to yield the deprotected prodrug. Its activity should be somewhat although not substantially less than the prodrug of Example 1, part (b.).

Example 6
Budesonide Galacturonide Prodrugs

(a.) Preparation of budesonide-21-yl methyl 2,3,4-tr-O-acetyl-β-D-galactosiduronate: Budesonide methyl acetyl

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galacturonide is prepared from budesonide as described in Example 2,
part (a.), but substituting methyl (tri-O-acetyl-α-D-
galactopyranosyl bromide)-uranate for methyl (tri-O-acetyl-α-D-
glucopyranosyl bromide)-uranate. The product may be isolated in the
same way as described for the product of Example 1, part (b.).

5   (b.) Preparation of budesonide-21-yl β-D-
galactosiduronate: Budesonide-β-D-galactosuronidate is prepared as
described in Example 2, part (b.), with respect to synthesis of
budesonide-β-D-glucuronidate, to yield the deprotected prodrug. Its
activity should be somewhat although not substantially less than the
10   prodrug of Example 2, part (b.).

Example 7
Other Prodrugs

The procedures of Examples 1, 2, 5 and 6 may be used
15   with other sugars such as chitobide, α-mannose and β-mannose to
yield other dexamethasone and budesonide prodrugs conjugated to
these sugars. Minor modifications in the synthetic method may be
necessary to accommodate sugar moieties other than glucuronic and
galacturonic acid, as may be readily deduced by those skilled in the
20   art. The procedures of Examples 1, 2, 5 and 6 may also be used with
respect to other corticosteroids such as betamethasone,
beclomethasone, prednisone, prednisolone, methyl prednisolone,
flunisolide, triamcinolone, acetonide, flucinolone acetonide,
flumethasone, chlorprednisone, fluprednisolone, 11-
25   deoxycorticosterone, 9α-fluorohydrocortisone, paramethasone and
dehydrocorticosterone.

Example 8
Studies in Human Stool Samples

The rate of release of corticosteroid from
30   corticosteroid prodrugs in human stool samples from patients with
ulcerative colitis was measured. The average % hydrolysis of
dexamethasone from dexamethasone-β-glucuronide was 25% in 5 hours at
37°C in undiluted, unhomogenized feces (conditions for these in
vitro hydrolysis studies are similar to those described above for
35   hydrolysis of prodrugs in rat intestinal contents and tissues). The
procedures used were the same as those used in Examples 3 and 4,
except that the samples were not homogenized, and were diluted
approximately 5-fold instead of 20-fold. Therefore, the time over
which drug will be released in the colon would be at least 20 hours
and probably longer. The stability of budesonide-β-glucuronide (20%
hydrolyzed in 5 hours at 37°C) indicated a minimum period of drug
release in the colon of 25 hours. Both these times for drug release
will allow drug to be delivered throughout the colon, including the

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descending and sigmoid colon based on average or accelerated transit times through the human colon.

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Claims

1.   A prodrug having the structural formula
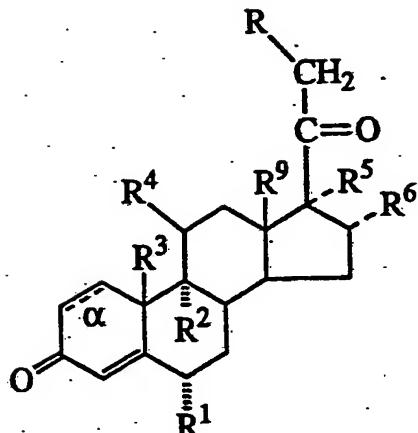
wherein:

R is a sugar moiety;

R^1 is selected from the group consisting of H, halogen and lower alkyl;

R^2 is selected from the group consisting of H and halogen;

R^3 is selected from the group consisting of H and lower alkyl;

R^4 is selected from the group consisting of OH and oxo;

R^5 is selected from the group consisting of H and OH;

R^6 is selected from the group consisting of H, lower alkyl or $-OCOR^7$ where R^7 is lower alkyl, or wherein R^5 and R^6 together form an $-O-R^8-O-$ bridge wherein R^8 is lower alkylene;

R^9 is selected from the group consisting of H and lower alkyl; and

α represents an optional double bond.

2.   The prodrug of claim 1, wherein R is a sugar moiety which is hydrolyzed by glycosidases present in the caecum and colon at a rate less than about 300 nmol/h/mg protein.

3.   The prodrug of claim 1, wherein R is selected from the group consisting of uronic acids, chitobide, α-mannose and β-mannose.

4.   The prodrug of claim 3, wherein R is a uronic acid having a structure selected from the group consisting of:
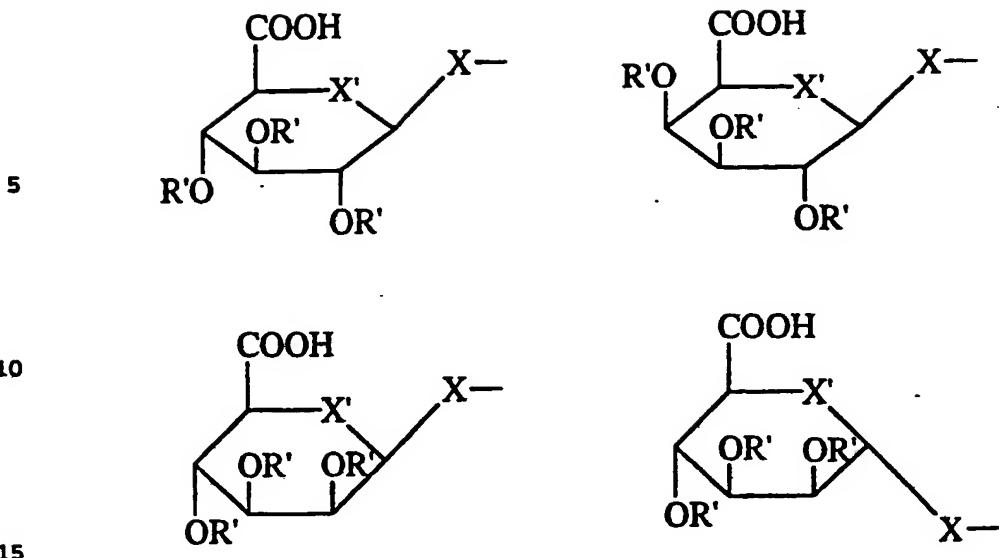
wherein:

X and X' are independently selected from the group consisting of oxygen and sulfur; and

R' is selected from the group consisting of hydrogen and lower alkyl.

5.   The prodrug of claim 1, wherein R is
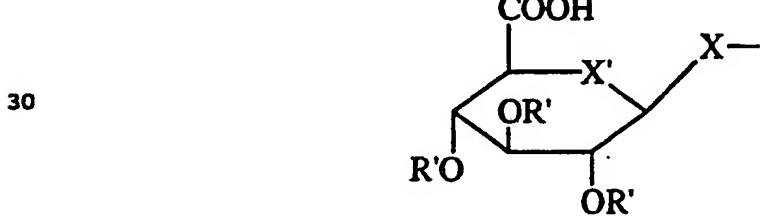
6.   The prodrug of claim 2, wherein the R' substituents are all hydrogen, X is oxygen and X' is oxygen.

7.   The prodrug of claim 1, wherein the corticosteroid is dexamethasone.

8.   The prodrug of claim 1, wherein the corticosteroid is betamethasone.

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9.   The prodrug of claim 1, wherein the corticosteroid is beclomethasone.

10.   The prodrug of claim 1, wherein the corticosteroid is budesonide.

11.   The prodrug of claim 1, wherein the corticosteroid is prednisolone.

12.   The prodrug of claim 1, wherein the corticosteroid is methyl prednisolone.

13.   The prodrug of claim 1, wherein the corticosteroid is flunisolide.

14.   A pharmaceutical composition comprising a therapeutically effective quantity of the prodrug of claim 1 in combination with a pharmaceutically acceptable carrier.

15.   The composition of claim 14, wherein the pharmaceutically acceptable carrier comprises a sterile aqueous solution.

16.   The composition of claim 14, which is in unit dosage form.

17.   The composition of claim 15, which is in unit dosage form.

18.   A method for delivering a corticosteroid drug to the colon of a warm-blooded host animal, which comprises orally administering to said host animal a therapeutically effective dose of the prodrug of claim 1.
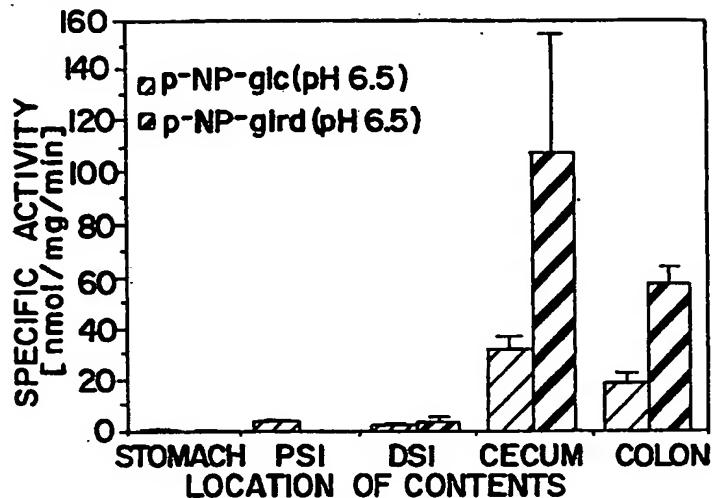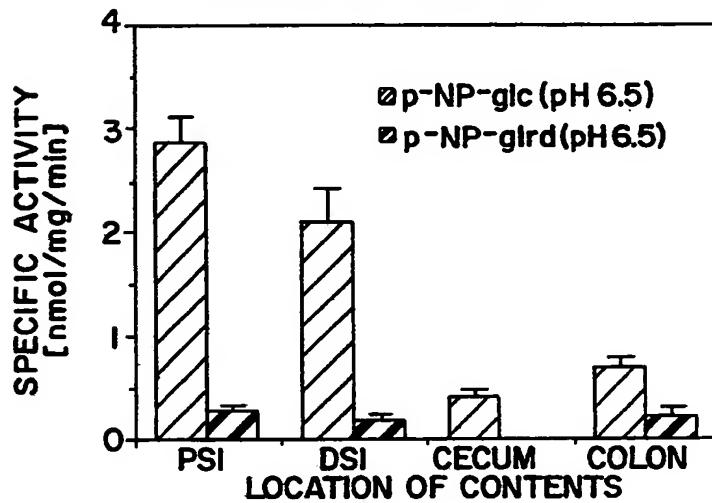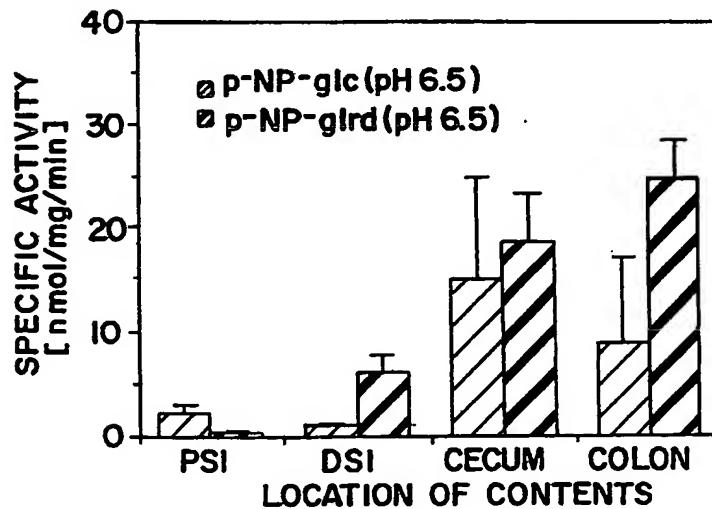
FIG.IA

FIG.IB

FIG.IC

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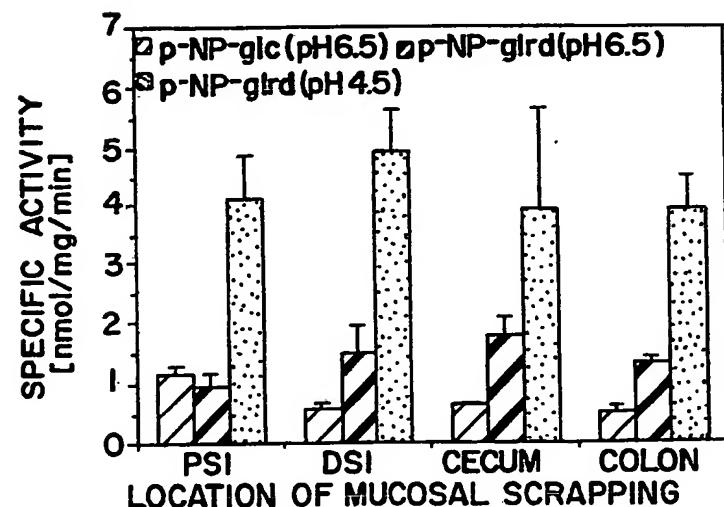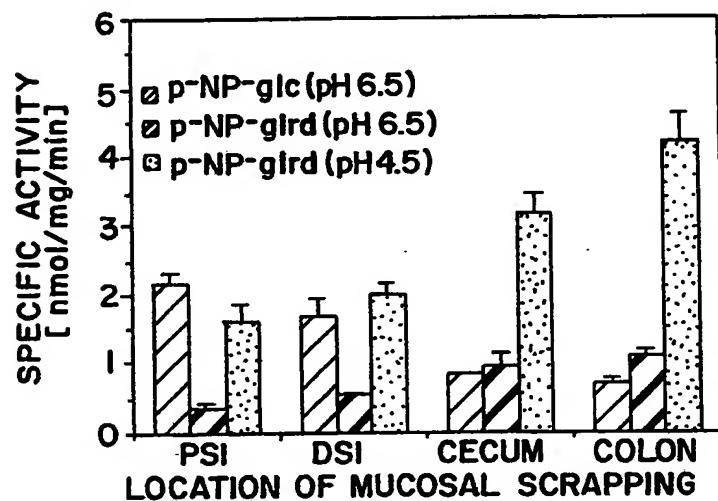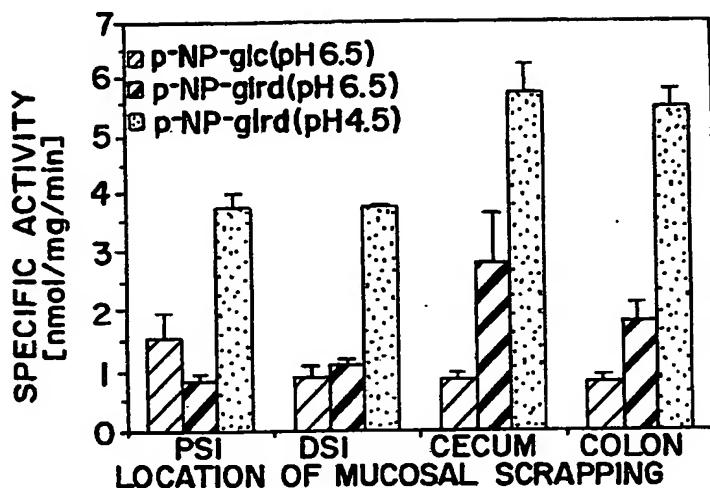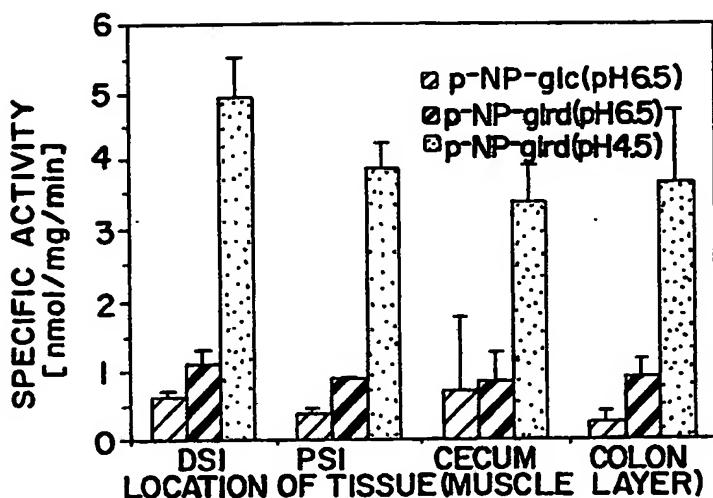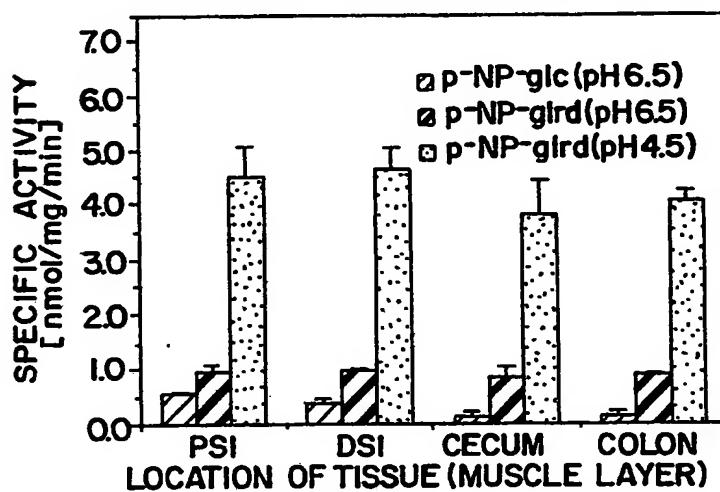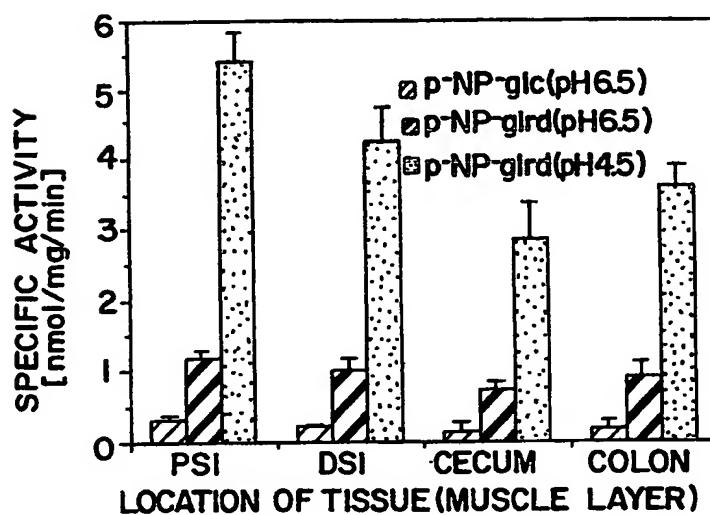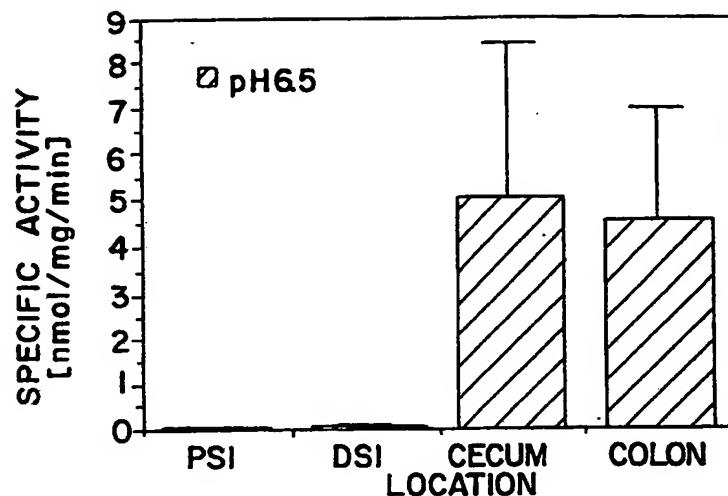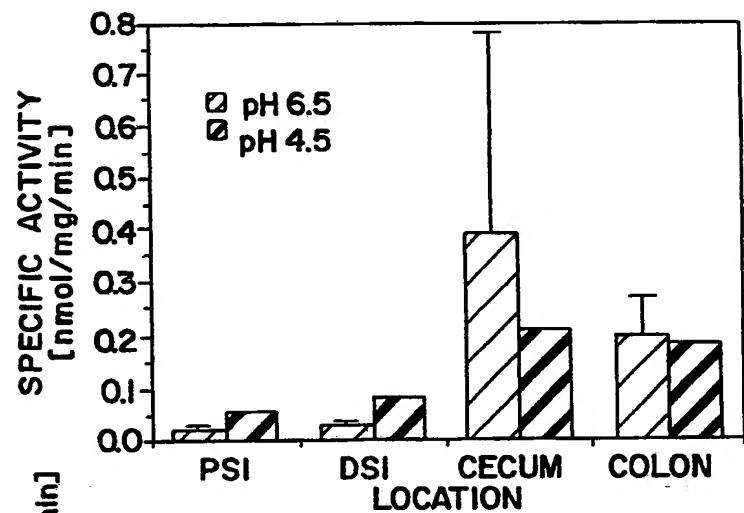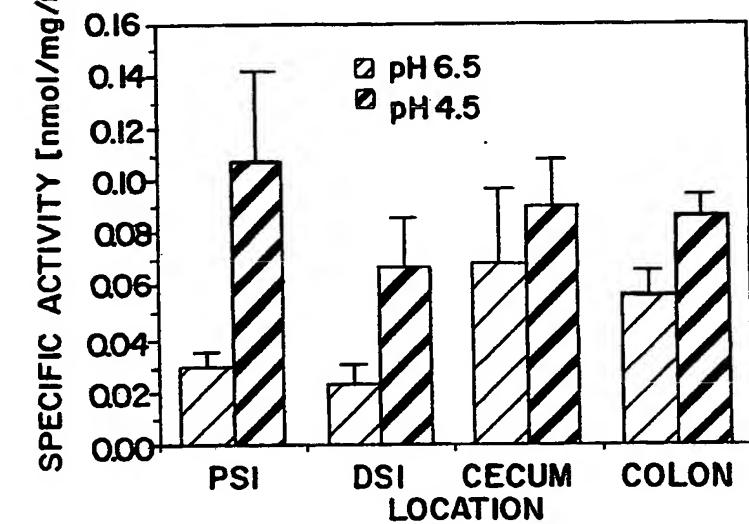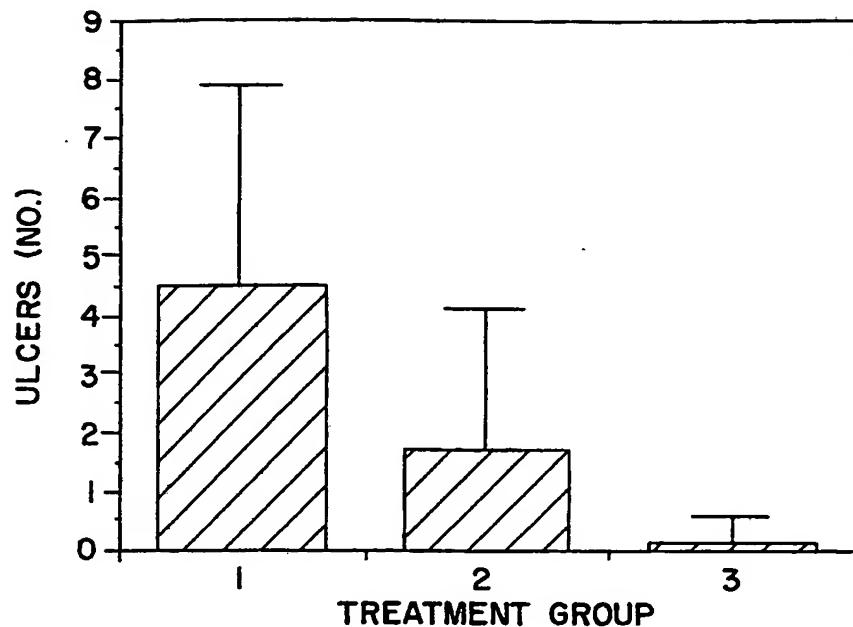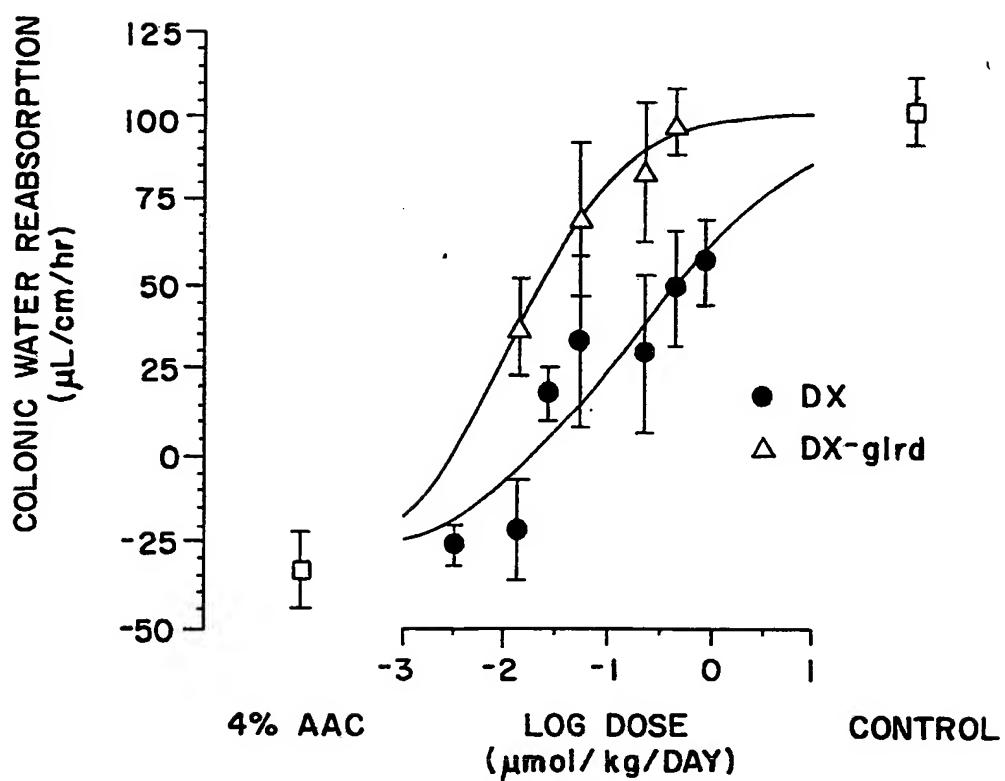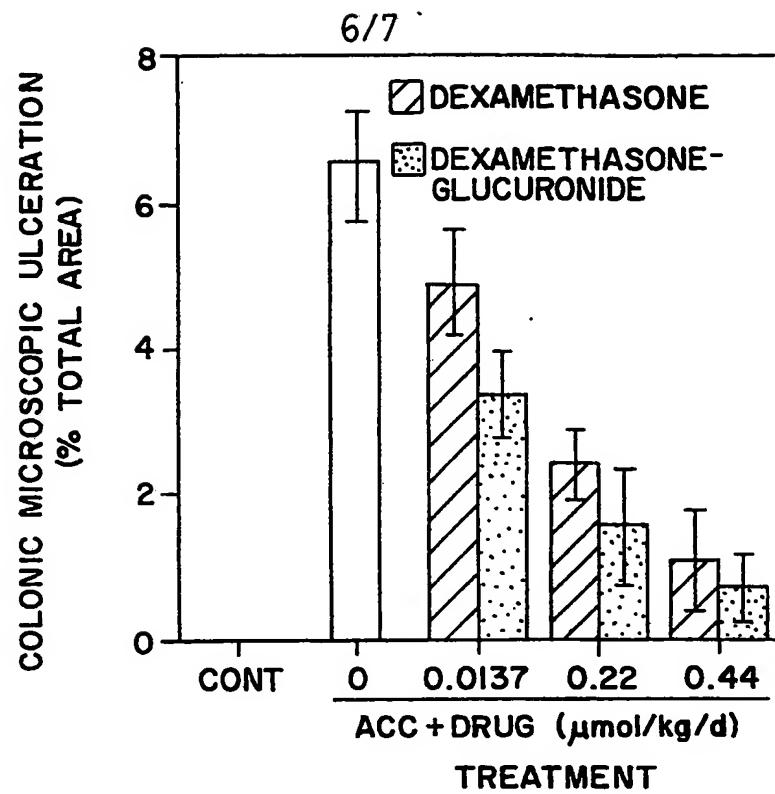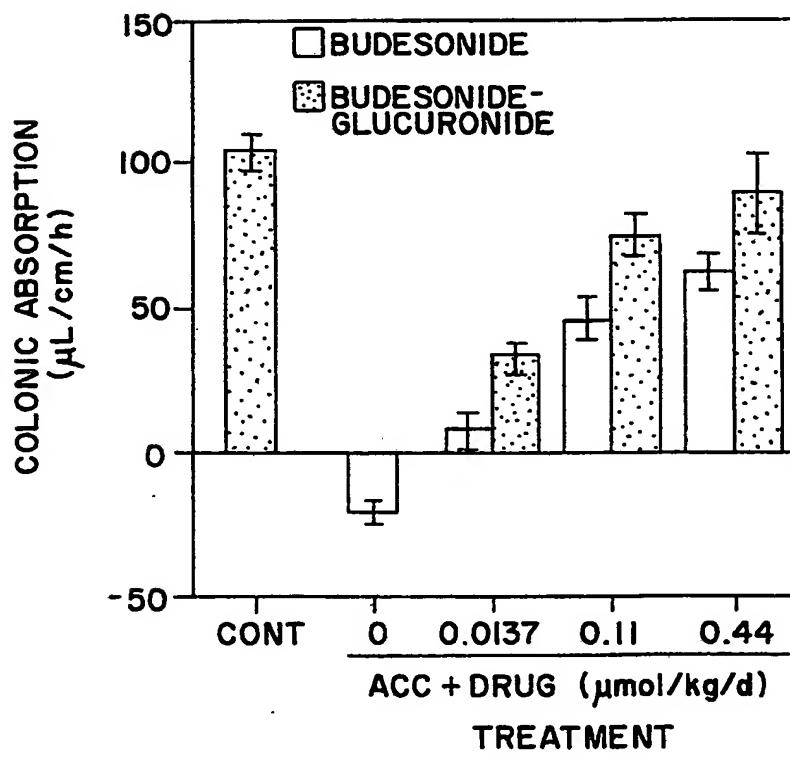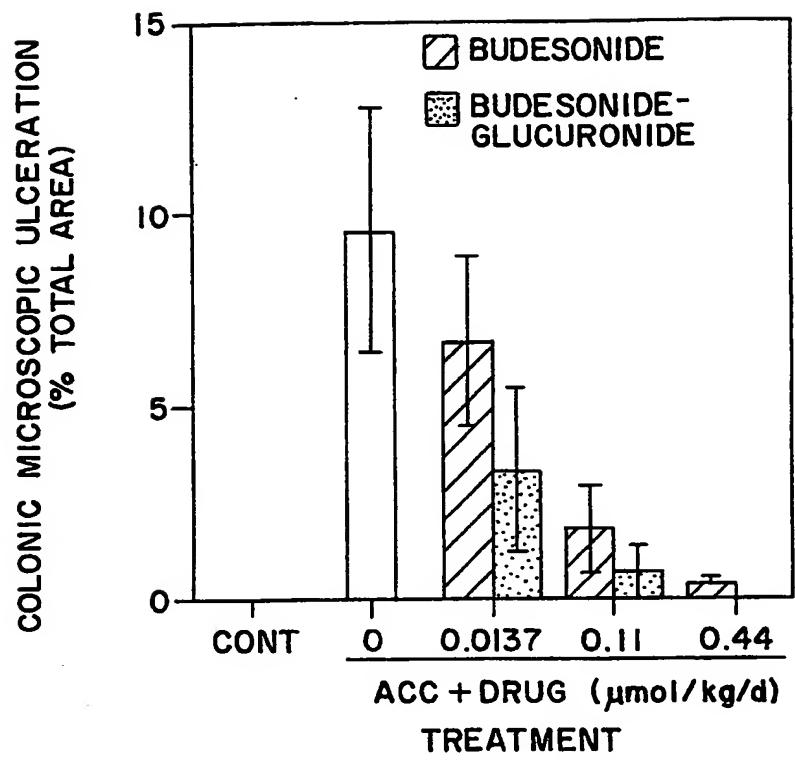
FIG.2A

FIG.2B

FIG.2C

FIG.3A

FIG.3B

FIG.3C

FIG.4A

FIG.4B

FIG.4C

FIG.5

FIG.6

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FIG. 7

FIG. 8

FIG.9

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[o]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5   C 07 J 17/00   C 07 J 71/00   A 61 K 31/58
A 61 K 31/70

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 J 17/00 C 07 J 71/00 A 61 K 31/00

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[o]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Biochemistry, vol. 8, no. 3, March 1969, (Washington, DC, US), V.R. MATTOX et al.: "Synthesis of C-21 glucosiduronates of cortisone and related corticosteroids", pages 1188-1199, see page 1189; figure 1	1,3-6.
X	GB,A,1015396 (MERCK & CO. INC.) 31 December 1965, see page 1, column 1, lines 13-37; column 2, lines 38-39; claims 1,28; examples	1,3-6, 11,12, 14,16, 18
X	EP,A,0123485 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 31 October 1984, see figure 1; claims 1,3,9,10; examples, & WO,A,8404041 25 October 1984 (cited in the application) -/-	1,2,7, 11,14, 18

[o] Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26-07-1993	14. 09. 93
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	C. MORENO

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Journal of Medicinal Chemistry, vol. 27, no. 1, January 1984, (Washington, DC, US), D.R. FRIEND et al.: "A colon-specific drug-delivery system based on drug glycosides and the glycosidases of colonic bacteria", pages 261-266, see page 262, scheme 1 (cited in the application)	1
X	Journal of Medicinal Chemistry, vol. 28, no. 1, January 1985, (Washington, DC, US), D.R. FRIEND et al.: "Drug glycosides: potential prodrugs for colon-specific drug delivery", pages 51-57, see page 51, scheme 1; page 52, table I (cited in the application)	1,2

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 9304202
SA   73850

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 01/09/93
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 1015396		None	
EP-A- 0123485	31-10-84	JP-T- 60501105	18-07-85
		WO-A- 8404041	25-10-84

EPO FORM P0479